**Title:**

Pectin-alginate does not further enhance exogenous carbohydrate oxidation in running.

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JFPB, JTG, GAW and JAB designed the research, JFPB, JT, AH, JTG and BN conducted the research, JFPB and JTG analyzed the data, JTG performed the statistical analysis, JFPB and JTG primarily wrote the paper and all authors read and approved the final version of the manuscript.

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## CONFLICTS OF INTEREST

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## ABSTRACT

**PURPOSE:** Maximizing carbohydrate availability is important for many endurance events. Combining pectin and sodium alginate with ingested maltodextrin-fructose (MAL+FRU+PEC+ALG) has been suggested to enhance carbohydrate delivery via hydrogel formation but the influence on exogenous carbohydrate oxidation remains unknown. The primary aim of this study was to assess the effects of MAL+FRU+PEC+ALG on exogenous carbohydrate oxidation during exercise compared to a maltodextrin-fructose mixture (MAL+FRU). MAL+FRU has been well established to increase exogenous carbohydrate oxidation during cycling, compared to glucose-based carbohydrates (MAL+GLU). However, much evidence focuses on cycling, and direct evidence in running is lacking. Therefore, a secondary aim was to compare exogenous carbohydrate oxidation rates with MAL+FRU *versus* MAL+GLU during running. **METHODS:** Nine trained runners completed two trials (MAL+FRU and MAL+FRU+PEC+ALG) in a double-blind, randomised crossover design. A subset (n=7) also completed a MAL+GLU trial to address the secondary aim, and a water trial to establish background expired $^{13}CO_2$ enrichment. Participants ran at 60% $\dot{V}O_2$peak for 120 min while ingesting either water only, or carbohydrate solutions at a rate of 1.5 g carbohydrate·min$^{-1}$. **RESULTS:** At the end of 120 min of exercise, exogenous carbohydrate oxidation rates were 0.9 (SD 0.5) g·min$^{-1}$ with MAL+GLU ingestion. MAL+FRU ingestion increased exogenous carbohydrate oxidation rates to 1.1 (SD 0.3) g·min$^{-1}$ ($p$=0.038), with no further increase with MAL+FRU+PEC+ALG ingestion (1.1 (SD 0.3) g·min$^{-1}$; $p$=1.0). No time x treatment interaction effects were observed for plasma glucose, lactate, insulin or non-esterified fatty acids, nor for ratings of perceived exertion or gastrointestinal symptoms (all $p$>0.05). **CONCLUSION:** To maximise exogenous carbohydrate oxidation during moderate-intensity running, athletes may benefit from consuming glucose(polymer)-fructose mixtures over glucose-based carbohydrates alone, but the addition of pectin and sodium alginate offers no further benefit.

## INTRODUCTION

Carbohydrate availability is a key determinant of endurance exercise performance. Low muscle and liver glycogen concentrations are strongly associated with fatigue during prolonged, moderate-to-high intensity exercise (1, 2). The ingestion of carbohydrate during exercise provides an additional (exogenous) source of carbohydrate, which can prevent or attenuate the decline in liver (3), and sometimes muscle (4, 5), glycogen contents. Increasing exogenous carbohydrate oxidation via altering the dose or type of carbohydrates ingested can improve endurance performance (6-9). Strategies to maximise the ability to digest, absorb and oxidise ingested carbohydrate are therefore a priority for endurance athletes during competition.

One well-established strategy for increasing exogenous carbohydrate oxidation rates during exercise, is the co-ingestion of glucose-fructose mixtures (10-12). When compared to glucose-based carbohydrates alone, isocaloric co-ingestion of fructose with glucose-based carbohydrates typically increases peak exogenous carbohydrate oxidation rates from ~1 $g \cdot min^{-1}$ to up to ~1.7 $g \cdot min^{-1}$ (13), which is thought to be (in part) due to fructose being absorbed by an additional intestinal transport route (GLUT5), and thereby bypassing the limiting step of intestinal glucose transport (primarily SGLT1)(14). A recent innovation in commercial carbohydrate sports drinks is the inclusion of pectin and sodium alginate alongside maltodextrin and fructose (15). When combined with water, this mixture can create a hydrogel upon exposure to a low pH environment such as the stomach (16). It is hypothesized that the hydrogel will allow for greater rates of gastric emptying via a reduction in nutrient sensing and thus increase intestinal carbohydrate delivery and absorption, thereby facilitating improvements in endurance performance (15). Whilst some evidence does indicate that the addition of pectin could accelerate gastric emptying during enteral feeding (17), other studies that have added either pectin to a meal (18) or alginate to meal preloads (19) demonstrate that each of these can *delay* gastric emptying at rest.

To date, only two studies have been conducted in which ingesting carbohydrate hydrogel has been compared to typical carbohydrate ingestion during exercise. These recent studies indicate no benefit to preloaded incremental time-to-exhaustion during running, or preloaded repeated sprint cycling performance with the ingestion of a maltodextrin-fructose-hydrogel, over maltodextrin-fructose alone (16, 20). It is possible, however, for hydrogels to only be relevant in specific contexts, such as when gastric emptying and carbohydrate availability are both contributing to limiting performance. This scenario may occur with high exercise intensities (>80% $\dot{V}O_2$peak), combined with a prolonged duration (>90 min), such as elite marathon racing. Methodological limitations mean that it is not yet possible to accurately assess exogenous carbohydrate oxidation at such intensities. Therefore, the current best approach to understand the physiology of carbohydrate hydrogels is likely to be to understand the metabolic responses at moderate-intensity exercise, combined with performance and gut comfort responses at race pace. This approach has been historically fruitful, as the primary principles of glucose-fructose mixtures were developed with data collected at moderate-intensity exercise (12), and have translated well into performances during high-intensity exercise (21). It is yet to be established whether a maltodextrin-fructose-hydrogel can enhance exogenous carbohydrate oxidation during exercise. It is also interesting to note that direct comparisons of exogenous carbohydrate oxidation from glucose plus fructose ingestion *versus* glucose alone have, to date, only been made during cycling-based exercise (13, 22). Given the substantial metabolic differences and the potential for differences in gastrointestinal function with the mechanical action of running compared to cycling (23), evidence derived from cycling cannot necessarily be extrapolated to running.

Therefore, the primary aim of the present study was to assess whether the addition of sodium alginate and pectin to a maltodextrin-fructose mixture enhances exogenous carbohydrate oxidation rates during running. A secondary aim was to assess whether a maltodextrin-fructose mixture enhances exogenous carbohydrate oxidation rates during running, when compared to isocaloric ingestion of

glucose-based carbohydrates alone. It was hypothesized that a maltodextrin-fructose mixture would enhance exogenous carbohydrate oxidation rates compared to maltodextrin-glucose ingestion, and that the addition of sodium alginate and pectin to a maltodextrin-fructose mixture would further increase exogenous carbohydrate oxidation rates.

## METHODS

### *Study design*

All participants completed preliminary testing followed by two main trials to address the primary aim, in a randomised, double-blind, crossover design separated by 7-14 days ($n$=9). During main trials, participants ingested a maltodextrin-fructose mixture either without (MAL+FRU), or with pectin and sodium alginate to create a hydrogel (MAL+FRU+PEC+ALG). Trials were conducted at the University of Bath, in accordance with the latest version of the Declaration of Helsinki and following institutional ethical approval (MSES 18/19-001). Participants provided informed written consent prior to participation. Randomisation was performed by JTG with online software (https://www.randomizer.org). Blinding and preparation of the test drinks was performed by an assistant who was not involved in the exercise tests.

Two subgroups of participants ($n$=7) also completed an additional trial with the ingestion of either glucose-based carbohydrates alone (MAL+GLU) or water alone (WATER) to address the secondary aim and to determine background $^{13}CO_2$ breath enrichment for calculation of exogenous carbohydrate oxidation rates, respectively.

### *Participants*

Ten trained male runners were recruited to the study (>1 year training in endurance running), but due to dropouts nine participants completed the two main trials (MAL+FRU and MAL+FRU+PEC+ALG), and seven participants completed the MAL+GLU and the WATER trial,

140 respectively (**Table 1**). Exclusion criteria included: metabolic or gastrointestinal disorders, smokers
141 or failure to pass a physical activity readiness questionnaire. Females were excluded on the rationale
142 of studying a homogenous population, since there are potential sex differences in gastric emptying
143 (24).
144
145 *Preliminary testing*
146 Participants' height (Leicester Height Measure, Seca GmbH, Hamburg, Germany) and mass (Tanita,
147 Tokyo, Japan) were measured. To determine running economy and peak oxygen consumption
148 ($\dot{V}O_2$peak), participants completed a graded exercise test to exhaustion on a motorised treadmill
149 (Ergo ELG70, Woodway, Weil am Rhein, Germany). Participants initially ran for 4 x 4 mins on a
150 0% gradient to establish the relationship between $O_2$ uptake and running speed (8-12 $km \cdot h^{-1}$) on a flat
151 treadmill. Following a 5-minute rest, participants then began the exhaustive test, whereby the
152 treadmill speed was fixed (at a speed based on participants perception in the 4-minute stages), and
153 the gradient was increased by 3% every 3 minutes, starting from a 1% gradient, until volitional
154 exhaustion. The running speed which elicited 60% $\dot{V}O_2$peak was interpolated and used for
155 prescribing running velocity during the experimental visits.
156
157 *Replication of usual diet and physical activity*
158 The approach to replication of usual diet and physical activity was based on the balance between
159 reducing day-to-day variability whilst minimizing participant burden (25). Participants recorded diet
160 and exercise for 2 days prior to the first experimental trial and replicated these prior to subsequent
161 trials. During this time, participants refrained from consuming foods with a high natural abundance
162 of $^{13}C$ to minimise background shifts in $^{13}C$ enrichment of expired gas arising from endogenous
163 carbohydrate stores being oxidized during exercise. For 24 h prior to each visit, participants refrained
164 from strenuous exercise, caffeine and alcohol. Participants also fasted for 8 h prior to each
165 experimental visit. Participants were reminded of these protocols 5 days and 3 days prior to trials.

Participants were also reminded of the fasting period 24 hours prior to trials. Adherence to these protocols was confirmed verbally with participants prior to each trial. This relatively modest method was thought to be appropriate for the current study design as the primary outcome measure (exogenous carbohydrate oxidation) has been shown to be unaffected by pre-exercise glycogen status (26), that would be influenced by dietary carbohydrate intake and physical activity levels.

*Main trials*

Participants arrived at the laboratory following pre-trial standardisation (confirmed by verbal questioning) and at a similar time of day within participants (±1 h). After a 5-min flush period (to washout dead space in tubing and familiarise participants), a 5-min sample of expired breath was taken using the Douglas bag method, and an additional breath sample was collected into an exetainer for analysis of $^{13}C$ enrichment. A cannula was then inserted into an antecubital vein and a resting blood sample was drawn. Participants then ran for 2 h at a speed eliciting 60% $\dot{V}O_2$peak. The run was performed in standard environmental conditions (17-22 °C dry bulb temperature, 40-65% relative humidity), and participants were fan cooled throughout.

*Carbohydrate drinks*

On all trials other than the WATER trial, participants ingested 140 mL of a 16% w/v solution upon initiating running, and then every 15 min until 105 min providing an average intake of 1.5 g carbohydrate·min$^{-1}$. The rate of carbohydrate intake was chosen to align with guidelines for prolonged exercise. As the solution concentration may affect the ability to form a hydrogel in the stomach this meant that fluid intake could not be tailored to expected sweat losses. This may have resulted in a slight hypohydration on all trials. The MAL+GLU drink provided 0.87 g maltodextrin·min$^{-1}$ and 0.63 g dextrose·min$^{-1}$, whereas both the MAL+FRU and MAL+FRU+PEC+ALG drinks provided 0.87 g maltodextrin·min$^{-1}$ and 0.63 g fructose·min$^{-1}$. The ratio of fructose/glucose to maltodextrin was dictated by that present in the commercially available product at the time of testing. Systematic

review indicates that a ratio closer to unity might be more optimal for balancing exogenous oxidation, gut comfort, and performance (14). MAL+GLU and MAL+FRU had 1 g sodium chloride·$L^{-1}$ added to match the MAL+FRU+PEC+ALG drink. Consistent with manufacturer's instructions, all drinks were made with low-calcium water (<40 mg·$L^{-1}$).

In order to quantify exogenous carbohydrate oxidation, carbohydrates with a high natural abundance of $^{13}C$ were used. The natural $^{13}C$ abundance of the MAL+GLU, MAL+FRU and MAL+FRU+PEC+ALG were -11.37, -11.20 and -11.86 δ‰ vs. Pee Dee Bellemnitella (PDB), respectively. Maltodextrin, dextrose (both MyProtein, Cheshire, UK) and fructose (PeakSupps, Bridgend, UK) were purchased as raw materials and mixed accordingly while the MAL+FRU+PEC+ALG, was purchased as a commercially available finished product (Maurten, Gothenburg, Sweden).

*Expired breath analysis*

Expired breath samples were analyzed using the Douglas bag method to establish rates of oxygen consumption and carbon dioxide production. At rest, a 5-min sample was collected after a 5-min equilibration period. During exercise, 1-min samples were taken after 1-min equilibration periods. Concurrently, ambient $O_2$ and $CO_2$ concentrations were measured to account for changes in inspired gas concentrations (27). Concentrations of $O_2$ and $CO_2$ were measured in a known volume of sample (Mini MP 5200, Servomex Ltd., Crowborough, UK), and the total volume of expired gas determined by evacuation using a dry gas meter (Harvard Apparatus, Holliston, USA). To determine $^{13}C$ enrichment of expired $CO_2$, breath samples were collected in 10 mL exetainers (Labco Ltd, Lampeter, UK), filled in duplicate by 10 s exhalation into a discard bag (Quintron Inc, Milwaukee, USA). At rest, participants exhaled for 20 s to ensure sufficient collection of expired gas.

Whole-body substrate oxidation was calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ according to stochiometric equations (28, 29). The $^{13}C/^{12}C$ ratio of expired $CO_2$ was determined by continuous flow isotope ratio mass spectrometry, and the enrichment expressed as δ per mil difference between the $^{13}C/^{12}C$ ratio of the sample and a known standard (30). The $\delta^{13}C$ was related to an international standard from which exogenous carbohydrate oxidation was calculated according to the following equation (31):

$$\text{Exogenous carbohydrate oxidation} = \dot{V}CO_2 \cdot \left(\frac{\delta Exp - \delta EXP_{bkg}}{\delta Ing - \delta Exp_{bkg}}\right)\left(\frac{1}{k}\right)$$

Where δExp is the $^{13}C$ enrichment of expired $CO_2$, δIng is the $^{13}C$ enrichment of the drink, and $\delta EXP_{bkg}$ is the $^{13}C$ enrichment of expired $CO_2$ during the WATER trial. For participants who did not complete a WATER trial, the group mean of the other participants was used for $\delta EXP_{bkg}$. *k* is the $\dot{V}CO_2$ with the oxidation of 1 g of glucose (0.7467 L $CO_2 \cdot g^{-1}$).

Some $^{13}C$ can be trapped within the bicarbonate pool with implications for the quantification of exogenous carbohydrate oxidation. However, during exercise, the increase in $CO_2$ production results in a rapid equilibration of expired $^{13}CO_2$ with the $^{13}CO_2/H^{13}CO_3^-$ pool and recovery of $^{13}CO_2$ from oxidation approaches 100% after 20 min of exercise at ~60 $\%\dot{V}O_2$peak (unpublished observations). Therefore, calculations on substrate oxidation were performed on data from 30 mins of exercise onwards.

*Blood sampling and analysis*

Venous blood samples (10 mL) were taken at rest and at 15, 30, 60, 90 and 120 min of exercise. Samples were collected into EDTA-containing tubes (Sarstedt, Germany) and centrifuged for 10 min at 4000 *g* and 4 ºC. Aliquots of plasma were stored at -80 ºC before analysis. Due to cost implications, only blood samples from the trials that related to the primary aim were analyzed (MAL+FRU and

MAL+FRU+PEC+ALG trials). Plasma was analyzed for glucose and lactate using an automated analyzer (RX Daytona, Randox, UK). Insulin (IBL International, Hamburg, Germany), and non-esterified fatty acid concentrations (NEFA, WAKO Diagnostics, Richmond, VA) were analyzed by ELISA and colorimetric assays, respectively. For all analyses, intra- and inter-assay coefficients of variation were below 10%.

*Subjective ratings*

Ratings of gastrointestinal distress were measured on a 7-point scale adapted from the Gastrointestinal Symptoms Rating Scale (GSRS; (32)). Four questions related to upper, three to central, and two to lower gastrointestinal symptoms. The GSRS has adequate internal consistence ($\alpha > 0.61$), construct and discriminant validity, and is suitable for comparisons over 6 weeks (32). Since these ratings are subjective and cannot therefore be readily compared between groups of people, only data for the primary comparison (MAL+FRU vs MAL+FRU+PEC+ALG) are presented.

*Statistical analysis*

An *a priori* sample size estimate was performed based on the effect size (Cohen's *d*) of exogenous carbohydrate oxidation rates in response to glucose-fructose co-ingestion compared to glucose alone based on the following equations:

$$d = \frac{\text{mean}_{\text{experimental}} - \text{mean}_{\text{control}}}{\text{SD}_{\text{pooled}}}$$

where

$$\text{SD}_{\text{pooled}} = \sqrt{\frac{(n_{\text{control}}-1)\text{SD}^2_{\text{control}} + (n_{\text{experimental}}-1)\text{SD}^2_{\text{experimental}}}{n_{\text{control}} + n_{\text{experimental}} - 2}}$$

Peak exogenous carbohydrate oxidation rates from glucose ingestion alone have been reported to be 1.06 (SD 0.11) $g \cdot min^{-1}$, compared to 1.75 (SD 0.31) $g \cdot min^{-1}$ with glucose-fructose co-ingestion (*n*

= 8, in a crossover design)(12). Using this effect size ($d$ = 2.49), 5 participants should provide power >95% to detect a difference with a two-tailed test and an α-level of 0.05. To ensure adequate power with the potential for dropouts, we aimed to recruit at least 7 participants.

Data were analyzed using Prism (v 8.2.1, GraphPad, San Diego, CA, USA) and SPSS (v24, IBM, Armonk, NY, USA). Data expressed over time (e.g. expired $^{13}CO_2$ enrichment, exogenous carbohydrate oxidation rates, $\dot{V}O_2$, $\dot{V}CO_2$, RER, plasma metabolite and hormone concentrations, RPE, and gastrointestinal symptom ratings) were analyzed by repeated measures ANOVA or mixed-effects model as appropriate. Summary statistics (e.g. peak exogenous carbohydrate oxidation rates, the percentage contribution of substrates to total energy expenditure) were analyzed by one-way ANOVA or two-tailed, paired *t*-tests with Bonferroni correction, as appropriate. An exploratory analysis was performed to assess whether baseline differences in NEFA concentrations were driving differences in whole-body substrate use by ANCOVA analysis on whole-body fat oxidation rates with baseline plasma NEFA concentrations as the covariate. Furthermore, data were checked for order effects by repeated measures ANOVA (trial order x time interaction) and one-way ANOVA (trial order) as appropriate. All data are expressed as means  (SD) in the text and tables, and as means ± 95%CI in figures, other than subjective data, which are presented as medians ± 95%CI. Differences were considered significant if $p \leq 0.05$.

## RESULTS

*Substrate oxidation and gas exchange*

No order effects were detected for either expired $^{13}CO_2$ enrichments (trial order: $p$ = 0.59; trial order x time interaction effect: $p$ = 1.0) or exogenous carbohydrate oxidation rates (trial order: $p$ = 0.61; trial order x time interaction effect: $p$ = 1.0). Furthermore, no order effects were detected for the total amount of fat ($p$ = 0.62), endogenous carbohydrate ($p$ = 0.38), or exogenous carbohydrate oxidised ($p$ = 0.93). Expired $^{13}CO_2$ enrichments increased during exercise (time effect, $p < 0.001$), and were

higher during MAL+FRU compared to MAL+GLU (treatment effect, $p < 0.001$, *post-hoc* comparison $p < 0.001$), with no further increase seen with MAL+FRU+PEC+ALG compared to MAL+FRU ($p = 0.11$; **Figure 1A**). Differences across time were detected between the WATER trial and the carbohydrate drink treatments (time x treatment interaction, $p < 0.001$). Exogenous carbohydrate oxidation rates increased over time (time effect, $p < 0.001$), and to a greater extent with both of the fructose-containing drinks compared to MAL+GLU (time x treatment interaction, $p < 0.001$; **Figure 1B**). At the end of exercise, exogenous carbohydrate oxidation rates were higher with MAL+FRU, compared to MAL+GLU ($p = 0.04$), but not further increased by MAL+FRU+PEC+ALG ($p = 1.0$). The exogenous oxidation rate expressed relative to ingestion rate at this timepoint equated to 59 (SD 19)%, 70 (SD 19)%, and 71 (SD 21)% with MAL+GLU, MAL+FRU MAL+FRU+PEC+ALG, respectively. Peak exogenous carbohydrate oxidation rates were 0.92 (SD 0.29) $g \cdot min^{-1}$, 1.08 (SD 0.26) $g \cdot min^{-1}$ and 1.11 (SD 0.31) $g \cdot min^{-1}$ with MAL+GLU, MAL+FRU MAL+FRU+PEC+ALG, respectively (all $p > 0.05$).

During MAL+GLU and MAL+FRU trials, fat oxidation was 234 (SD 50) $kcal \cdot h^{-1}$ and 165 (SD 83) $kcal \cdot h^{-1}$ respectively ($p = 0.14$). Fat oxidation was 255 (SD 120) $kcal \cdot h^{-1}$ during the MAL+FRU+PEC+ALG trial, which was higher than MAL+FRU ($p = 0.04$). During MAL+GLU and MAL+FRU trials, endogenous carbohydrate oxidation was 525 (SD 89) $kcal \cdot h^{-1}$ and 530 (SD 99) $kcal \cdot h^{-1}$ respectively ($p = 0.93$). During the MAL+FRU+PEC+ALG endogenous carbohydrate oxidation was lower compared to MAL+FRU (434 (SD 112) $kcal \cdot h^{-1}$, $p = 0.05$). During MAL+GLU, exogenous carbohydrate oxidation was 165 (SD 60) $kcal \cdot h^{-1}$. MAL+FRU increased exogenous carbohydrate oxidation to 201 (SD 66) $kcal \cdot h^{-1}$ ($p = 0.05$), with no further increase from MAL+FRU+PEC+ALG ingestion (193 (SD 66) $kcal \cdot h^{-1}$; $p = 0.66$).

When expressed as the contribution to total energy expenditure, fat oxidation contributed ~20-25% of total energy expenditure during MAL+GLU and MAL+FRU trials and increased to ~30% of total

energy expenditure during MAL+FRU+PEC+ALG ($p$ = 0.02; **Figure 2**). However, this increase in fat oxidation as a contribution to total energy expenditure between MAL+FRU and MAL+FRU+PEC+ALG (mean difference: 10.7%, 95%CI: 0.2 to 21.1%), did not remain after baseline NEFA concentrations were added as a covariate (adjusted mean difference: 7.8%, 95%CI: -0.6 to 16.1%, $p$ = 0.07). Endogenous carbohydrate oxidation contributed ~60% of total energy expenditure during MAL+GLU and MAL+FRU trials, and decreased to ~50% of total energy expenditure during MAL+FRU+PEC+ALG ($p$ = 0.03; **Figure 2**). Exogenous carbohydrate oxidation contributed ~18% of total energy expenditure during MAL+GLU, and increased to ~22% of total energy expenditure during MAL+FRU ($p$ = 0.05; **Figure 2**). Exogenous carbohydrate oxidation was not further increased with MAL+FRU+PEC+ALG compared to MAL+FRU ($p$ = 0.71; **Figure 2**).

$\dot{V}O_2$, $\dot{V}CO_2$ and RER all displayed main effects of time (all $p < 0.05$), but no treatment effects were detected (all $p > 0.29$; $p = 0.08$ for RER), and no differences over time were detected (time x treatment interaction effects, all $p > 0.45$; **Figure 3**).

*Plasma insulin and metabolite concentrations*

Plasma glucose, lactate and insulin concentrations all rose slightly at the onset of exercise (time effect for all, $p < 0.01$), to a similar extent across time in both MAL+FRU and MAL+FRU+PEC+ALG trials (treatment effect and time x treatment interaction, all $p > 0.20$; **Figures 4A, 4B and 4C,** respectively). Plasma NEFA concentrations were ~0.13 $mmol \cdot L^{-1}$ higher at baseline in the MAL+FRU+PEC+ALG trial compared to the MAL+FRU trial ($p$ = 0.03; **Figure 4D**). During exercise, plasma NEFA concentrations declined (time effect, $p < 0.001$), to a similar level across time in both trials (treatment effect and time x treatment interaction, both $p$ = 0.12).

*Subjective ratings*

RPE, upper, central and lower gastrointestinal symptom ratings all increased throughout exercise (time effect, all $p < 0.01$), to a similar extent across time in both trials (treatment effect and time x treatment interaction, all $p > 0.07$; **Figures 5A, 5B, 5C and 5D**, respectively).

## DISCUSSION

The present data demonstrate that, when ingesting carbohydrates at 90 g per hour during running, the addition of pectin and sodium alginate to ingested glucose-fructose does not further enhance exogenous carbohydrate oxidation rates, when compared to a glucose-fructose mixture alone. However, ingestion of glucose-fructose mixture can enhance exogenous carbohydrate oxidation during running, when compared to isocaloric ingestion of glucose-based carbohydrates alone.

Maximizing carbohydrate availability during exercise is a key goal for many endurance athletes (22). A novel nutrient blend of sodium alginate and pectin, combined with a maltodextrin-fructose mixture has recently been developed, and has been proposed to further enhance exogenous carbohydrate oxidation during exercise (15). This combination purports to produce a hydrogel when exposed to the acidic environment of the stomach, thereby encapsulating the carbohydrate (15). It is expected that this hydrogel may attenuate the reduction in gastric emptying rates seen with large amounts of carbohydrate ingestion, thereby facilitating high exogenous carbohydrate oxidation rates during exercise. To the best of the authors' knowledge, there are currently only two randomised, controlled trials that have examined the effects of co-ingesting pectin and sodium alginate with carbohydrates during exercise. Both of these studies demonstrated no changes in whole-body metabolism, ratings of gut discomfort or perception of effort, or performance during running (16), or cycling (20). Consistent with this, we also observed no differences in ratings of gut discomfort or perception of effort. However, it is possible that increased exogenous carbohydrate availability above that seen with maltodextrin-fructose mixtures only enhances performance during very specific contexts. Therefore, further insight about the potential for this nutritional strategy to influence performance

could be gained from establishing whether pectin and sodium alginate co-ingestion with carbohydrate affects exogenous carbohydrate oxidation.

In the present study, exogenous carbohydrate oxidation rates were not further increased by the co-ingestion of pectin and sodium alginate with a maltodextrin-fructose mixture, compared to a maltodextrin-fructose mixture alone. If the mechanism by which pectin and alginate are proposed to enhance carbohydrate delivery is via accelerating gastric emptying, then the lack of effect on exogenous carbohydrate oxidation is perhaps not surprising, as gastric emptying rates are not thought to be limiting to exogenous carbohydrate oxidation when large amounts of carbohydrate are ingested during exercise (33). These data demonstrate that there is no increase in exogenous carbohydrate availability with the co-ingestion of alginate and pectin with a maltodextrin-fructose mixture, and thereby can explain why recent studies have demonstrated a lack of effect on endurance performance (16, 20).

It is well-established that the co-ingestion of fructose with glucose can enhance exogenous carbohydrate oxidation rates during cycling-based exercise, when compared to the co-ingestion of glucose-based carbohydrates alone (13, 34). However, the ability to extrapolate findings from cycling to other modes of exercise is uncertain. When compared to cycling, running typically results in higher rates of fat oxidation and a concomitant decrease in whole-body carbohydrate oxidation rates (35, 36). Furthermore, running is thought to pose a greater mechanical stress on the gastrointestinal system, potentially altering the capacity for intestinal absorption and thus limiting the rate of digestion, absorption and oxidation of exogenous carbohydrate (35). Nevertheless, the only direct comparison to date of prolonged running *versus* cycling reported equivalent exogenous carbohydrate oxidation rates with the ingestion of a glucose-fructose mixture (35). However, in that study, participants exercised at the same relative intensity during both trials (60% $\dot{V}O_2$peak), resulting in a ~5% higher absolute exercise intensity (based on oxygen consumption and energy expenditure) with

396 running *versus* cycling (35). The higher absolute energy cost of exercise could have driven a higher
397 exogenous carbohydrate oxidation rate in the running trial and offset any potential reduction in
398 exogenous carbohydrate oxidation rates seen with running. Therefore, whilst the present data
399 demonstrate that a glucose-fructose mixture can increase exogenous carbohydrate oxidation during
400 running, it remains to be established whether running *versus* cycling alters the efficiency or capacity
401 for digestion, absorption and oxidation of exogenous carbohydrate.
402
403 Unexpectedly, during the trial where pectin and sodium alginate were co-ingested with a
404 maltodextrin-fructose mixture, we observed a higher rate of fat oxidation compared to ingestion of a
405 maltodextrin-fructose mixture alone. Since there was no change in exogenous carbohydrate
406 oxidation, this resulted in a reduction in endogenous carbohydrate oxidation. It is tempting to
407 speculate that this could be a direct effect of the test drink. For example, it has been suggested that
408 hydrogels may attenuate nutrient-sensing in the proximal gastrointestinal tract (15), which would
409 result in higher gastric emptying rates and lower insulin secretion (37). However, plasma insulin
410 concentrations were unaffected by the addition of pectin and sodium alginate to carbohydrate in the
411 present study. Additionally, a baseline difference was observed in plasma NEFA concentrations,
412 which was higher in the MAL+FRU+PEC+ALG trial. Elevated baseline NEFA is one possible
413 explanation for the higher whole-body fat oxidation in that trial (38). Indeed, when baseline NEFA
414 concentrations are added as a covariate, the difference in fat oxidation between trials is no longer
415 statistically significant. The reasons for this baseline difference in NEFA concentrations are not clear.
416 Whilst participants were asked to replicate diet and activity in the days before trials, this was only
417 checked by verbal confirmation, and it is possible that this was not fully adhered to. Differences in
418 carbohydrate intake and/or physical activity levels could have caused baseline glycogen
419 concentrations to be lower in the MAL+FRU+PEC+ALG trial. Fortunately, this is unlikely to have
420 implications for our primary and secondary aims, as exercising with low glycogen contents does not
421 alter exogenous carbohydrate oxidation rates (26). This highlights the importance of considering pre-

422 trial standardization with respect to the specific aims and methods of a study. If a study design
423 requires tighter control of pre-exercise carbohydrate availability, then researchers should consider
424 requesting participants to report back on the accuracy of diet and physical activity replication and/or
425 provide food packages to facilitate adherence (25).
426
427 A potential limitation with the present study is that it was not confirmed whether the addition of
428 pectin and sodium alginate to carbohydrate resulted in hydrogel formation within the stomach or
429 therefore altered gastric emptying. Nevertheless, the product was made accordingly to manufacturer's
430 instructions, and this method has been recently shown to produce a hydrogel within a low pH
431 environment *in vitro* (16). Furthermore, the measurement of exogenous carbohydrate oxidation
432 encapsulates the integrated sum of gastric emptying, intestinal absorption and oxidation of the
433 ingested carbohydrate. Therefore, if a carbohydrate hydrogel is to enhance carbohydrate delivery and
434 thereby performance, an increase in exogenous carbohydrate oxidation is most likely a requirement.
435 Whilst the study was powered for the outcome of exogenous carbohydrate oxidation with the
436 specified comparisons, the relatively small sample size has the potential to be underpowered for some
437 of our other outcome measures reported. Inadequate power for some outcomes has the potential to
438 result in either a type II error (false negative), but also overestimate the true effect size when an effect
439 is detected. It should also be acknowledged that the exercise intensity employed in the present study
440 is not relevant to elite-level marathon running, which occurs at ~90% $\dot{V}O_2$peak (39). Given the
441 differences in gastric emptying rates at high- *versus* moderate-intensity exercise (40), it is not possible
442 to directly extrapolate the findings of the present study to exercise intensities above ~80% $\dot{V}O_2$peak.
443 However, the measurement of exogenous carbohydrate oxidation also becomes problematic at high
444 exercise intensities, and therefore it is unlikely that measurements of exogenous carbohydrate
445 oxidation can be made at elite-level marathon race with the current methods available.
446

In conclusion, when carbohydrates are ingested at rates recommended for prolonged endurance-type exercise (*i.e.* 90 grams per hour), maltodextrin-fructose mixtures increase exogenous carbohydrate oxidation compared to the ingestion of glucose-based carbohydrates alone. The additional ingestion of pectin and sodium alginate with a maltodextrin-fructose mixture does not further increase exogenous carbohydrate oxidation, or alter the perception of effort or ratings of gastrointestinal symptoms during moderate-intensity running. Given the technical difficulties in assessing exogenous carbohydrate oxidation at exercise intensities reflective of elite marathon racing, decisions on the use of hydrogels in elite sport should be based on the total balance of evidence from mechanistic studies at moderate-intensity exercise, performance studies at race pace, combined with careful observations in elite athletes during hard training and racing.

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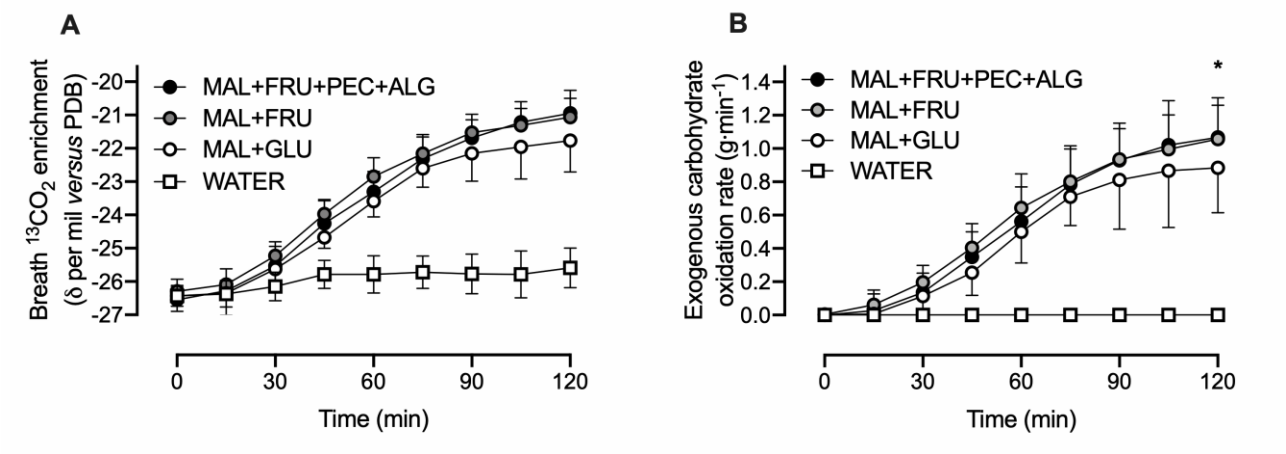
570 **Figure legends**

571 **Figure 1**. Breath $^{13}CO_2$ enrichment (A), and exogenous carbohydrate oxidation rates (B) during 120
572 min of running at 60% $\dot{V}O_2$peak with the ingestion of water (WATER; *n*=7), or 1.5 g·min$^{-1}$ of
573 carbohydrate in the form of maltodextrin plus glucose (MAL+GLU; *n*=7), maltodextrin plus fructose
574 (MAL+FRU; *n*=9), or maltodextrin plus fructose with pectin and sodium alginate
575 (MAL+FRU+PEC+ALG; *n*=9). Data are means (error bars: 95%CI). **p*<0.05 for MAL+GLU *versus*
576 MAL+FRU.

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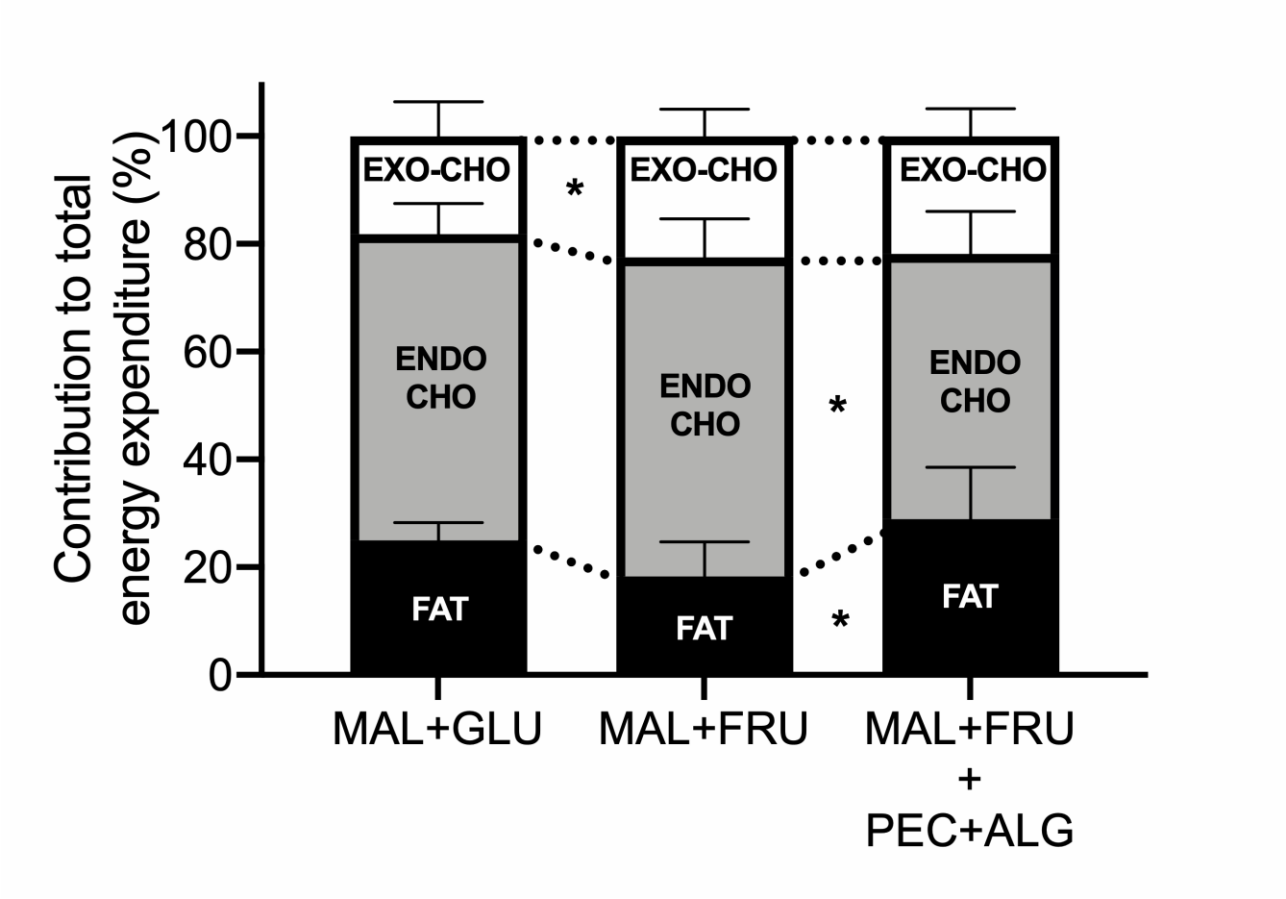
583 **Figure 2**. Whole-body fat (FAT), endogenous carbohydrate (ENDO CHO) and exogenous
584 carbohydrate oxidation rates (EXO CHO) during 120 min of running at 60% $\dot{V}O_2$peak with the
585 ingestion of 1.5 g·min$^{-1}$ of carbohydrate in the form of maltodextrin plus glucose (MAL+GLU; *n*=7),
586 maltodextrin plus fructose (MAL+FRU; *n*=9), or maltodextrin plus fructose with pectin and sodium
587 alginate (MAL+FRU+PEC+ALG; *n*=9). Data are means (error bars: 95%CI). *$p$<0.05 for differences
588 between treatments. Data were calculated from minutes 30-120 of exercise.

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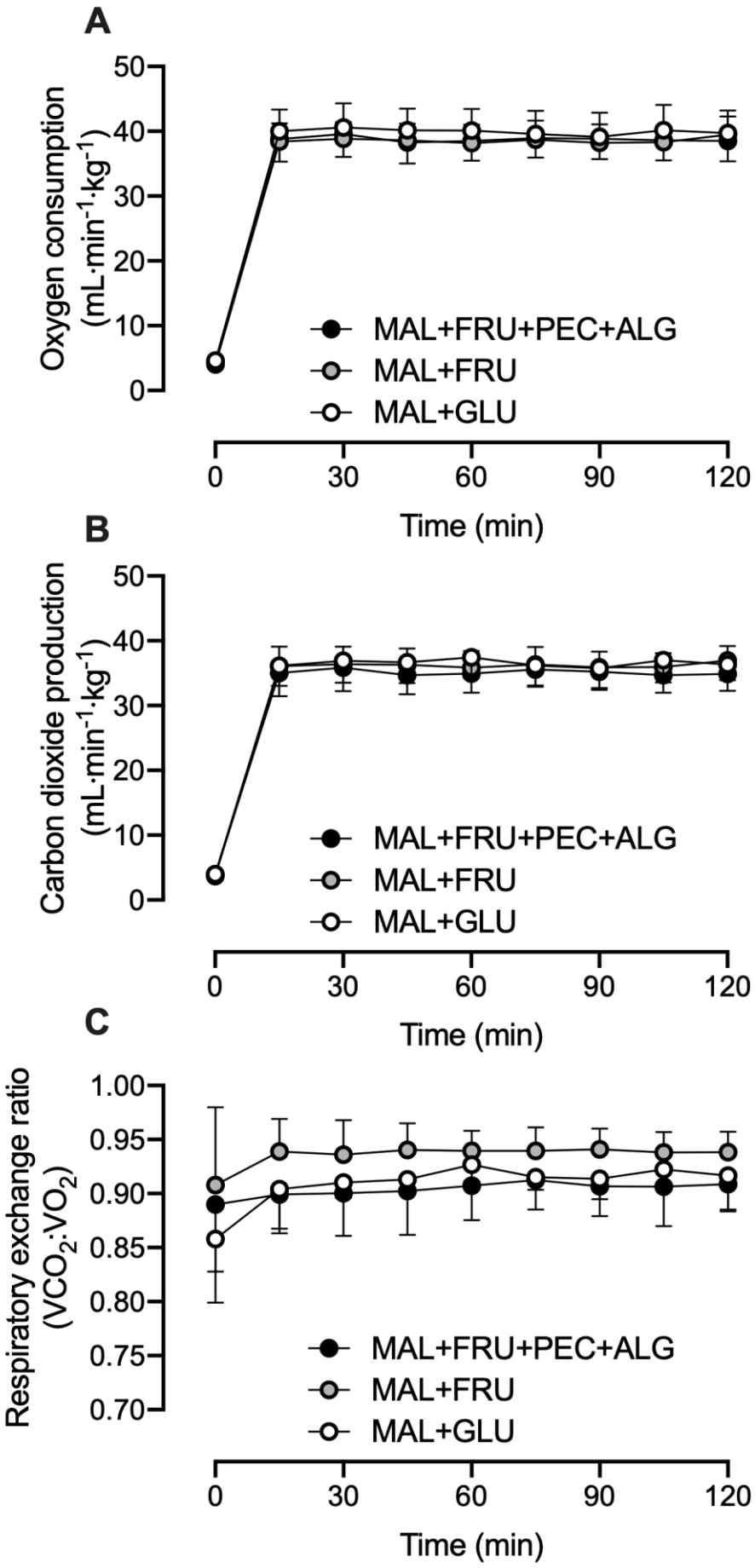
591 **Figure 3**. Oxygen consumption (**A**), carbon dioxide production (**B**), and respiratory exchange ratio
592 (**C**) during 120 min of running at 60% $\dot{V}O_2$peak with the ingestion of 1.5 g·min$^{-1}$ of carbohydrate in
593 the form of maltodextrin plus glucose (MAL+GLU; *n*=7), maltodextrin plus fructose (MAL+FRU;
594 *n*=9), or maltodextrin plus fructose with pectin and sodium alginate (MAL+FRU+PEC+ALG; *n*=9).
595 Data are means (error bars: 95%CI).


A
Oxygen consumption (mL·min-1·kg-1)
50
40
30
20
10
0
MAL+FRU+PEC+ALG
MAL+FRU
MAL+GLU
0
30
60
90
120
Time (min)
B
Carbon dioxide production (mL·min-1·kg-1)
50
40
30
20
10
0
MAL+FRU+PEC+ALG
MAL+FRU
MAL+GLU
0
30
60
90
120
Time (min)
C
Respiratory exchange ratio (VCO2:VO2)
1.00
0.95
0.90
0.85
0.80
0.75
0.70
MAL+FRU+PEC+ALG
MAL+FRU
MAL+GLU
0
30
60
90
120
Time (min)

596

**Figure 4**. Plasma glucose (**A**), lactate (**B**), insulin (**C**), and non-esterified fatty acid (NEFA; **D**) concentrations during 120 min of running at 60% $\dot{V}O_2$peak with the ingestion of 1.5 g·min$^{-1}$ of carbohydrate in the form of, maltodextrin plus fructose (MAL+FRU; *n*=9), or maltodextrin plus fructose with pectin and sodium alginate (MAL+FRU+PEC+ALG; *n*=9). Data are means (error bars: 95%CI). *$p$<0.05 for MAL+FRU *versus* MAL+FRU+PEC+ALG.

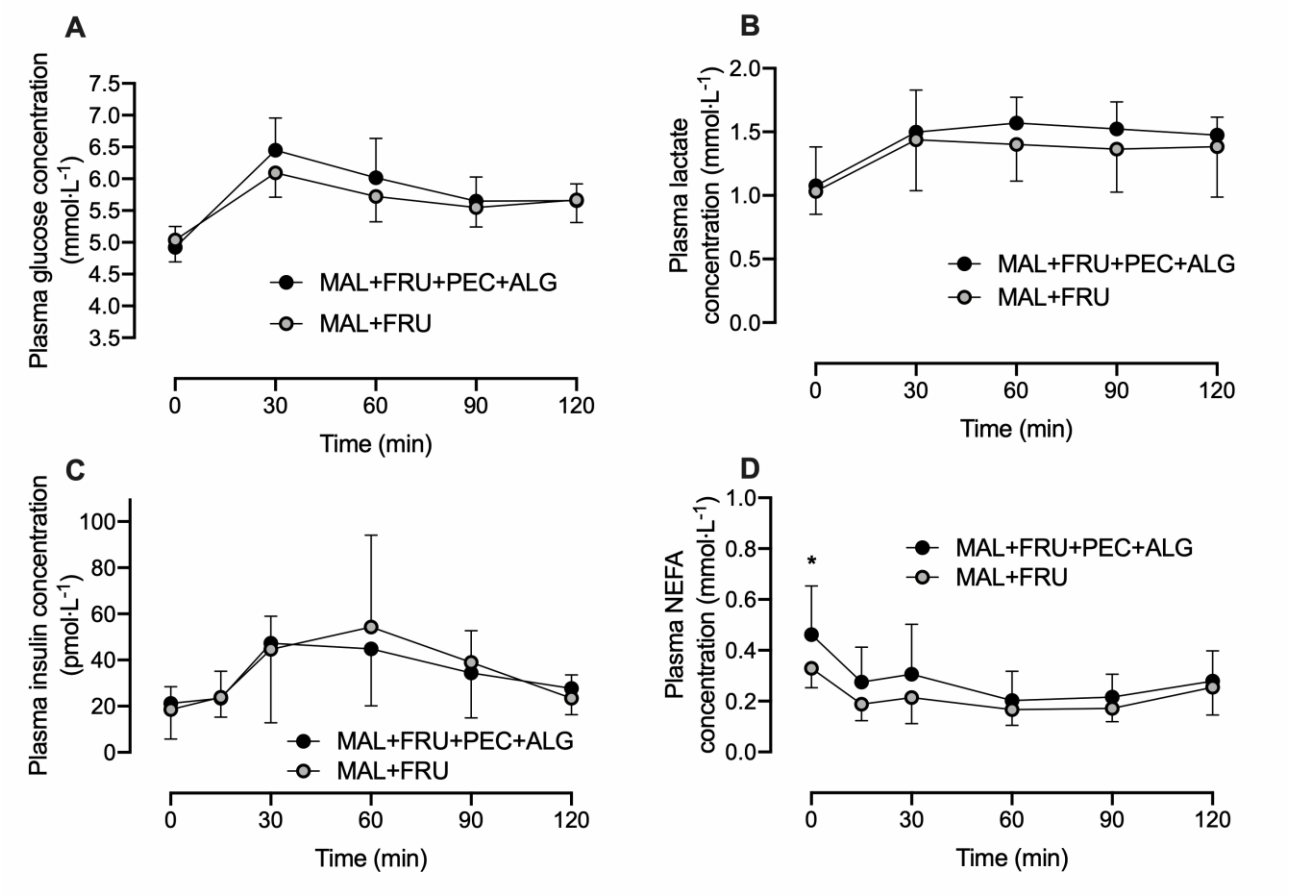
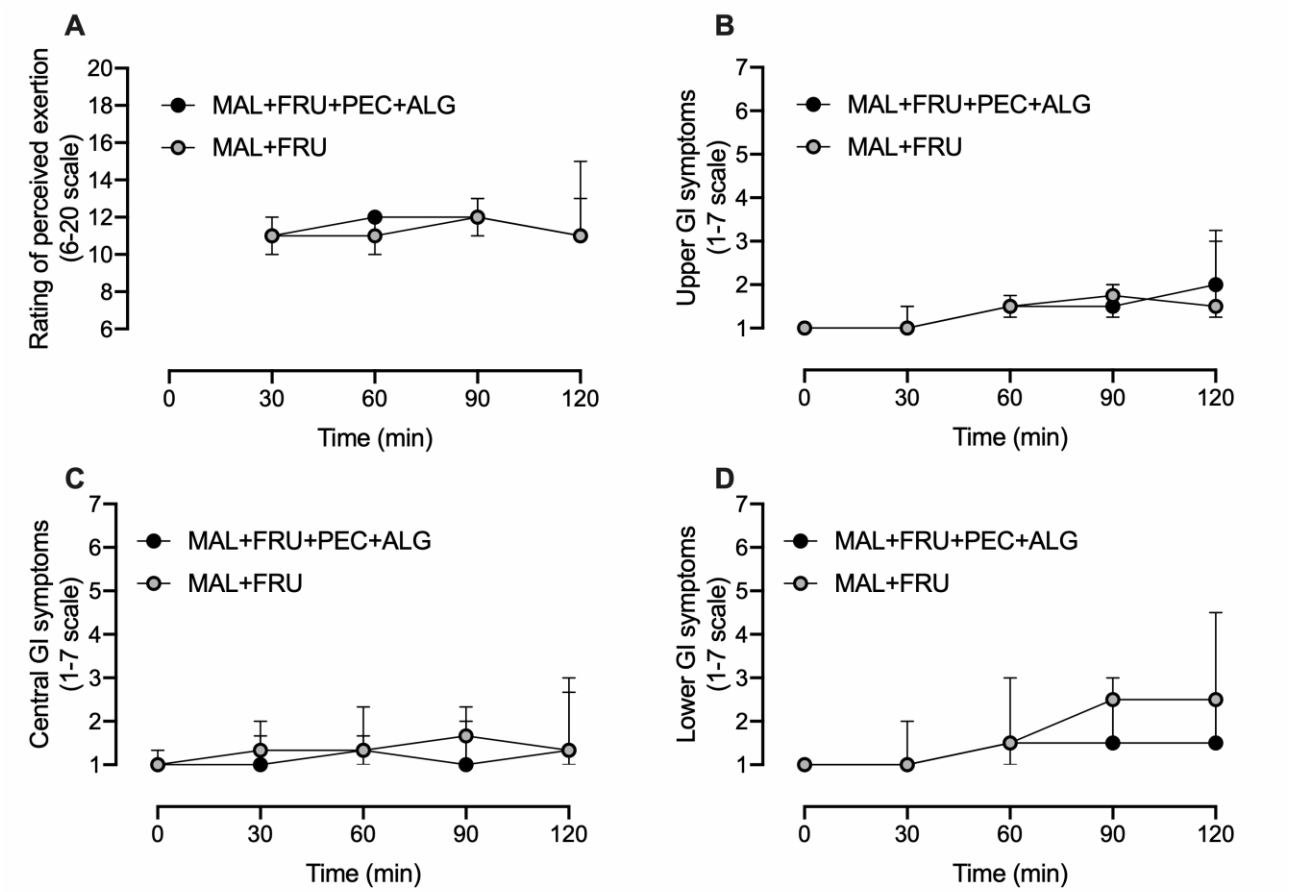


**Figure 5**. Ratings of perceived exertion (**A**), upper (**B**), central (**C**), and lower (**D**) gastrointestinal (GI) symptoms during 120 min of running at 60% $\dot{V}O_2$peak with the ingestion of 1.5 g·min$^{-1}$ of carbohydrate in the form of, maltodextrin plus fructose (MAL+FRU; *n*=9), or maltodextrin plus fructose with pectin and sodium alginate (MAL+FRU+PEC+ALG; *n*=9). Data are medians (error bars: 95%CI).



612 **Table 1. Participant characteristics.**

| | **Characteristics** |
|---|---|
| Age | 22 (18-30) years |
| Body mass | 69 (61-74) kg |
| Height | 1.82 (1.74-1.88) m |
| $\dot{V}O_2$peak | 63 (56-72) $mL \cdot min^{-1} \cdot kg^{-1}$ |
| Running speed to elicit 60% $\dot{V}O_2$peak | 10.7 (9.3-11.8) $km \cdot h^{-1}$ |

613 Data are means (ranges). $\dot{V}O_2$peak, peak oxygen consumption.

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